INFECTION AND IMMUNITY, June 1983, p. 1155–1162
0019-9567/83/061155-08$02.00/0

Vol. 40, No. 3

Lysosomal Response of a Murine Macrophage-Like Cell Line Persistently Infected with *Coxiella burnetii*

EMMANUEL T. AKPORIAYE, JAMES D. ROWATT, ADAM A. ARAGON, AND OSWALD G. BACA*

Department of Biology, The University of New Mexico, Albuquerque, New Mexico 87131

Received 27 January 1983/Accepted 15 March 1983

The lysosomal response of a murine macrophage-like tumor cell line (J774) during persistent infection with *Coxiella burnetii* was examined. By using acid phosphatase as a lysosomal marker, it was shown that phagosome-lysosome fusion occurred in J774 cells persistently infected with *C. burnetii*. This observation was verified using thorium dioxide, an electron-dense compound that is sequestered in secondary lysosomes. The phagolysosomes contained viable replicating rickettsiae. Spectrofluorometric analysis indicated that the phagolysosomal pH of persistently infected cells was acidic. In attempts to correlate rickettsial survival with lysosome function, the activities of several lysosomal enzymes were assayed in both infected and uninfected cells. Activities of acid phosphatase and β-acetylglucosaminidase were not significantly altered during infection. However, infected cells appeared to display slightly higher intracellular lysozyme, β-glucuronidase, and β-galactosidase activities.

Phagocytes, including macrophages and polymorphonuclear leukocytes, play a key role in the control of infectious agents. Through the participation of toxic oxygen metabolites and perhaps in concert with lysosomes, macrophages and polymorphonuclear leukocytes kill and degrade ingested microorganisms (12, 17, 23). Lysosomal involvement in microbial destruction has been proposed to be dependent on phagosome-lysosome (P-L) fusion (3, 19, 21, 23) which exposes ingested microorganisms to the acid environment of the lysosome and also to a complement of lysosomal hydrolases. Despite this armamentarium for microbial destruction, certain facultative and obligate parasites survive within phagocytes. Different strategies are employed by such ingested parasites to ensure their survival and subsequent proliferation. For example, *Mycobacterium tuberculosis* and *Toxoplasma gondii* owe their survival within cultured macrophages to the prevention of P-L fusion (1, 19). *Trypanosoma cruzi* and *Rickettsia tsutsugamushi* establish infection in macrophages and polymorphonuclear leukocytes, respectively, only after escaping from the phagosome into the cytoplasm (24, 26, 31). Although they allow P-L fusion, however, *Mycobacterium lepraemurium* and *Salmonella typhimurium* are refractory to lysosomal enzymes (10, 18).

The role of lysosomal enzymes in the killing of ingested microorganisms is still unclear. It has been suggested that these enzymes may participate only in the degradation of killed bacteria (1, 2). The finding that activated macrophages with enhanced microbicidal capacity contain significantly increased quantities of lysosomal hydrolases (11, 20) suggests a possible role for lysosomal enzymes in microbial destruction.

In this communication we report the lysosomal response of a murine macrophage cell line (J774) during persistent infection with the obligate intracellular parasite *Coxiella burnetii*. We conclusively show that lysosomes fuse with *C. burnetii*-containing vacuoles. We also present evidence that the rickettsia-containing vacuoles exhibit an acid pH and that although the activities of some lysosomal enzymes appear to increase slightly during infection, the activities of others remain unchanged.

MATERIALS AND METHODS

***C. burnetii* propagation and purification.** Phase I *C. burnetii*, Nine Mile strain, obtained from R. A. Ormsbee (Rocky Mountain Laboratory, U.S. Public Health Service, Hamilton, Mont.), was used in these studies. The organisms were cloned by Ormsbee and Peacock (28), propagated in our laboratory in mouse fibroblasts (L-929), and purified by differential centrifugation as previously described (4). The purified organisms were suspended in Dulbecco balanced salt solution (pH 7.4), and the rickettsial concentration was determined by the method of Silberman and Fiset (33). Rickettsial suspensions were stored at −70°C until required.

Maintenance and infection of J774 cells. The J774 cells used in this study were cultured in Dulbecco modified Eagle medium (DMEM) with 10% fetal calf serum. The cells were grown in a static suspension culture in plastic petri dishes at 37°C in a 10% CO_2

atmosphere. The cultures were passaged every 3 days, at which time cell viability was determined by the dye (erythrosin B) exclusion technique (29). The macrophage-like cells (2×10^4 cells per ml) were exposed to phase I *C. burnetii* at a parasite-to-cell ratio of 500:1. Gimenez-stained (14) cells were examined periodically for the presence or absence of cell-associated rickettsiae.

Electron microscopy. Samples were prepared for electron microscopy by standard techniques. Sedimented material was fixed in 3% glutaraldehyde prepared in 0.1 M cacodylate buffer (pH 7.4) and containing 1% sucrose. The fixed material was washed in buffer and postfixed in 1% cacodylate-buffered OsO_4. Pellets were washed and then dehydrated through an alcohol series into propylene oxide. The cells were embedded in Spurr medium and sectioned with a Porter-Blum ultramicrotome. Sections were placed on specimen grids, stained with aqueous uranyl acetate and lead citrate, and examined with either an AE1 Corinth 275 or a 6B transmission electron microscope.

Thorotrast labeling. Thorotrast (thorium dioxide; Polysciences, Inc., Warrington, Pa.) labeling of secondary lysosomes was performed essentially by the method of Jones and Hirsch (19). J774 cells were allowed to take up thorotrast (1:40 dilution) for 18 h while held in plastic petri dishes in DMEM containing 10% fetal calf serum. The cells were maintained at 37°C in a 4% CO_2 atmosphere. At the end of the 18-h incubation period, the cells were washed three times in incomplete DMEM (without 10% fetal calf serum) to eliminate excess unphagocytized thorotrast. Fresh complete medium (DMEM containing 10% fetal calf serum) was added, and the cells were cultured for an additional 2 h to allow most of the thorotrast to localize in lysosomes (1). Cells were exposed to *C. burnetii* (500 rickettsiae per cell) and cultured for 48 h in the presence of the parasites at 37°C in a 4% CO_2 atmosphere. Cells already heavily infected with *C. burnetii* (31 days postexposure to rickettsiae) were similarly labeled with thorotrast. The cells were fixed and processed for electron microscopy as described above. Thin sections were stained only with aqueous uranyl acetate before electron microscopic examination.

Acid phosphatase activity. The presence of the lysosomal enzyme marker acid phosphatase was determined histochemically by the Gomori technique (7, 15), using beta-glycerophosphate (grade III; Sigma Chemical Co., St. Louis, Mo.) as the enzyme substrate and lead as the capture agent (15). J774 cells heavily infected with *C. burnetii* (41 days postexposure to rickettsiae) were examined. For comparative purposes, control uninfected cells were also assessed for acid phosphatase activity. Cells were fixed for 30 min in 3% glutaraldehyde in 0.2 M cacodylate buffer, pH 7.4, containing 1% sucrose. After fixation, cells were washed in three changes (10 min each) of cold cacodylate buffer and then incubated for 1 h at 37°C in a shaker water bath with constant agitation (100 rpm) in the reaction medium buffered to pH 5.0 with Tris-maleate. After incubation, the cells were washed with three changes of Tris-maleate (pH 5.0) and then postfixed for 1 h in 1% OsO_4 buffered in cacodylate. Control experiments involved the omission of the substrate from the reaction medium. (When examined by electron microscopy, such control cells were devoid of the electron-dense reaction product.) Cells were processed for electron microscopy as described above. Before examination, sections were double stained with aqueous uranyl acetate and lead citrate.

Lysosomal enzyme assays. Enzyme assays were performed using control uninfected and *C. burnetii*-infected J774 cells. Cells were washed in phosphate-buffered saline, pH 7.4, and suspended to a concentration of 5×10^6 cells per ml. The cell suspension was subjected to sonic treatment for 30 s with a Branson S75 Sonifier, and the resulting homogenate was used directly for enzymatic analyses. Acid phosphatase activity was determined in a final volume of 1 ml in 0.12 M acetate buffer (pH 5.0) containing 16 mM β-nitrophenyl phosphate (9). The reaction was terminated by the addition of 3 ml of 1.25 N NaOH, and the absorbancy was read at 420 nm. β-Glucuronidase activity was measured by the method of Fishman et al. (13), using 0.01 M phenolphthalein glucuronide as the substrate. The final reaction volume was 1 ml in 0.1 M acetate buffer (pH 4.5). The reaction was stopped with 2.5 ml of alkaline glycine buffer (pH 10.5), and the absorbancy was measured at 540 nm. β-Acetylglucosaminidase and β-galactosidase activities were determined as described by Beck and Tappel (5) in 1.5 ml of McIlvaine citrate-phosphate buffer (pH 4.2 for β-acetylglucosaminidase and pH 3.0 for galactosidase) containing 2.4 mM β-nitrophenyl-*N*-acetyl-β-D-glucosaminide or 1 mM β-nitrophenyl-β-D-galactopyranoside, respectively. Reactions were stopped with 1.5 ml of 1.25 N NaOH, and the absorbancy was read at 420 nm. Lysozyme activity was assayed spectrophotometrically by the method of Nerurkar (25) in homogenates of control uninfected and *C. burnetii*-infected J774 cells.

Protein concentrations were determined by the method of Bradford (6).

All enzyme substrates were purchased from the Sigma Chemical Co.

Measurement of intralysosomal pH. Approximately 6×10^4 cells in DMEM were dispensed into petri dishes (35 by 10 mm), each containing a cover slip (11.5 by 30 mm). Cells were cultured until a monolayer was formed. Fluorescein isothiocyanate-labeled dextran (Sigma Chemical Co.) at a concentration of 1 mg/ml was incubated with the culture medium for 24 h in a 10% CO_2 atmosphere (27). Fluorescence measurements were made by the method of Ohkuma and Poole (27) in a Mark I spectrofluorometer (Farrand Optical Co., Inc., New York, N.Y.). Cover slips with cell monolayers were washed three times at 37°C, to remove unincorporated fluorescein isothiocyanate-labeled dextran in a solution containing only the salts, glucose, and pyruvate of DMEM. To minimize the effect of light reflections, cover slips were placed in a cuvette containing the above solution and aligned at a 30° angle to the excitation beam (27). The intensities of fluorescence at 495- and 450-nm excitation wavelengths were measured; the emission wavelength was 519 nm. The ratio of fluorescence at 495 nm to that at 450 nm (495/450 ratio) was determined and compared with a standard curve. The standard curve was prepared using a solution of fluorescein isothiocyanate-labeled dextran (1 μg/ml) in modified DMEM. The ratio of fluorescence intensities at 495 and 450 nm was determined at pHs ranging from 3.5 to 7.0 (0.5-pH-unit intervals). The 495/450 ratios versus pH were plotted to generate the standard curve.

RESULTS

Labeling of secondary granules with thorotrast. Thorotrast labeling was performed on infected J774 cells either after a prolonged infection or after short-term exposure (48 h) to *C. burnetii*. Figure 1 shows a J774 cell which had been exposed to *C. burnetii* for 48 h in the culture medium and later labeled with thorotrast. The electron-dense marker was detected in phagosomes and rickettsia-containing vacuoles. Only a few rickettsiae were ingested during the exposure period, as evidenced by the presence of an average of two rickettsiae per infected cell. Heavily infected cells processed at 31 days postexposure to *C. burnetii* (Fig. 2) also demonstrated the thorotrast label within rickettsia-containing vacuoles and membrane-bound vesicles which may be phagosomes or phagolysosomes. Cell cytoplasm and other organelles revealed a distinct lack of the thorotrast marker. Figure 2 also shows a secondary granule (lysosome) delivering its thorotrast contents into a vacuole containing rickettsiae.

Acid phosphatase cytochemistry. To unequivocally demonstrate that P-L fusion occurred in cells infected with *C. burnetii*, acid phosphatase, a lysosomal enzyme marker, was localized histochemically. Figure 3 is of a heavily infected cell (41 days postexposure to *C. burnetii*) displaying substantial amounts of the electron-dense reaction product (lead phosphate) in a rickettsia-containing vacuole. Although most of the rickettsiae observed were intact, a few were noted to be in various states of degradation. As expected, control uninfected cells also displayed substantial acid phosphatase activity (Fig. 4).

Enzyme assays. Following the observation that P-L fusion occurs in rickettsia-infected J774 cells, we postulated that survival of *C. burnetii*

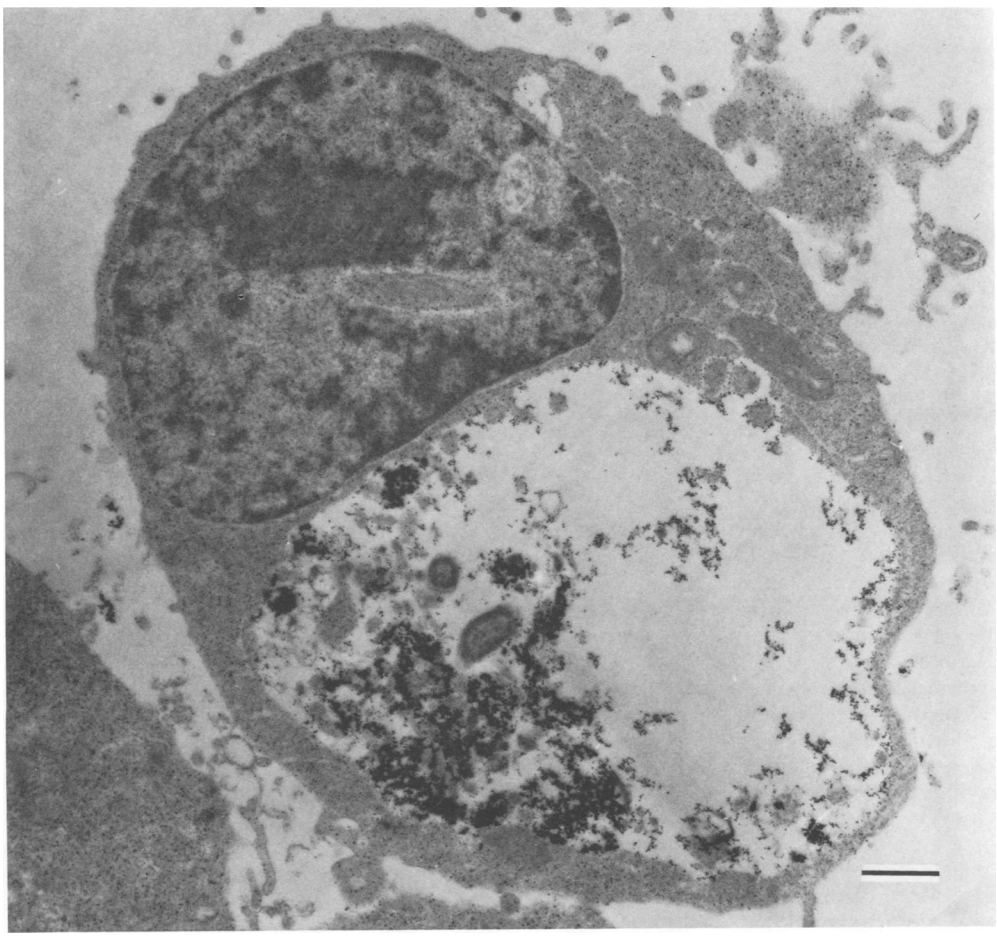

FIG. 1. Electron micrograph of a J774 cell at 48-h postexposure to phase I *C. burnetii* showing electron-dense thorotrast particles within a rickettsia-containing vacuole. Bar = 0.97 μm.

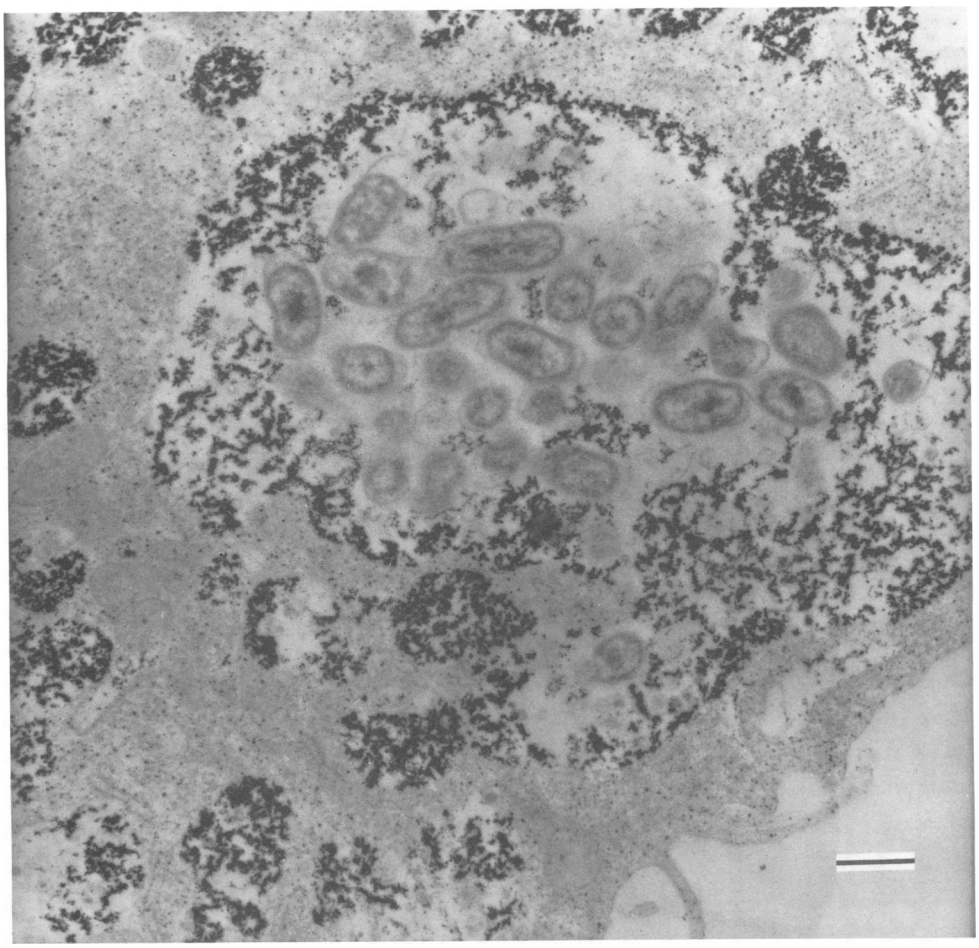

FIG. 2. Electron micrograph of a phase I *C. burnetii*-infected J774 cell cytoplasm showing labeling of secondary granules with thorotrast. The thorotrast marker is also present within a rickettsia-containing vacuole. Note a secondary granule emptying its thorotrast and contents into the vacuole containing rickettsiae. Cells had been infected for 31 days. Bar = 1.55 μm.

might correlate with a concomitant decrease in the activities of lysosomal hydrolases. Enzyme activities were expressed on a per cell basis and not on a per milligram of protein basis because of the large number of intracellular rickettsiae that would haved skewed the results. On a per cell basis, activities of acid phosphatase and β-acetylglucosaminidase were not significantly altered during infection (Table 1). However, infected cells appeared to display slightly higher lysozyme, β-glucuronidase, and β-galactosidase activities.

There was no significant difference between the phagolysosomal pH of control uninfected cells (5.14 ± 0.05; four determinations) and that of heavily infected cells (5.21 ± 0.07; four determinations). That fluorescence-derived pH measurements were primarily due to rickettsia-containing phagolysosomes was indirectly supported by UV light microscopy which showed fluorescein isothiocyanate-labeled dextran-specific green fluorescence primarily in the large rickettsia-containing vacuoles.

DISCUSSION

Based on the cytochemical demonstration of acid phosphatase activity within rickettsia-containing vacuoles, Burton et al. (7, 8) suggested that P-L fusion occurred in *C. burnetii*-infected L-929 mouse fibroblasts and monkey kidney (Vero) cells. Our study extends and confirms these observations in another cell type. In the present study, we have demonstrated (i) the localization of the electron-dense tracer thorium dioxide in lysosomes, (ii) acid phosphatase activity within rickettsia-containing vacuoles, and

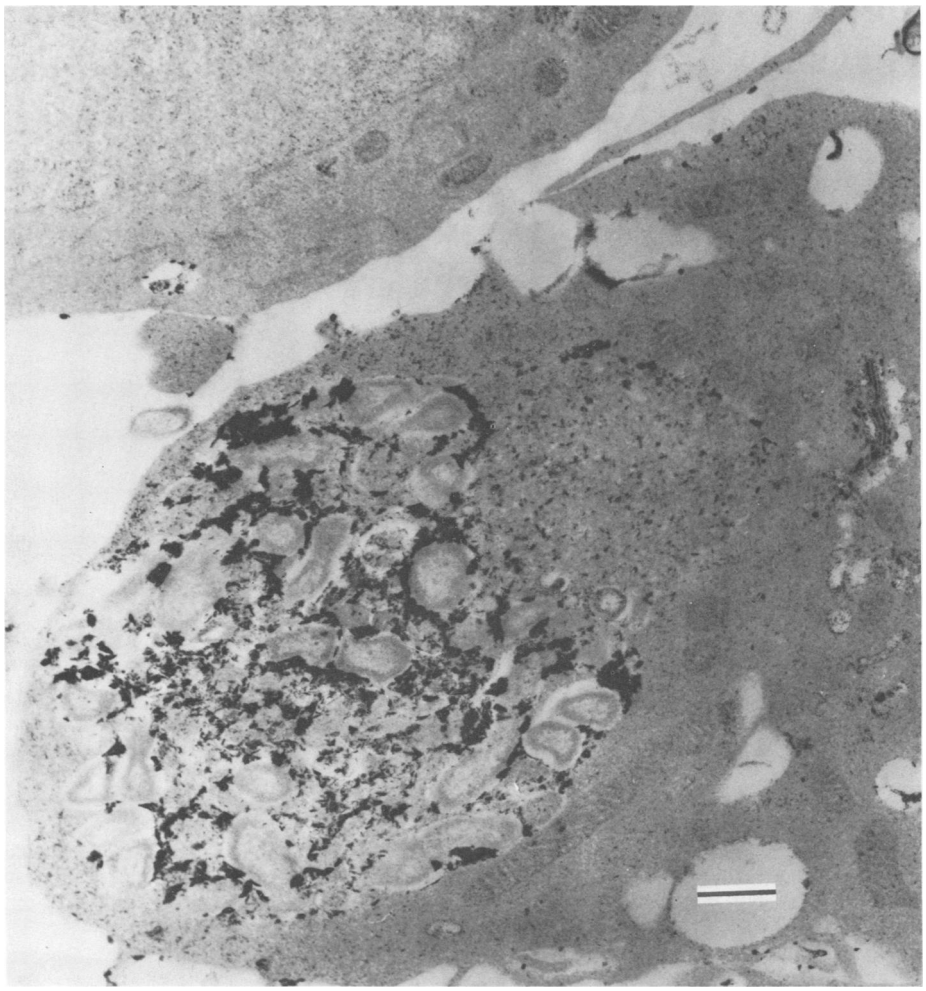

FIG. 3. Acid phosphatase activity in a J774 cell infected with phase I *C. burnetii* (41 days postexposure). A substantial electron-dense lead phosphate reaction product is present within the rickettsia-containing vacuole and in association with the rickettsiae. Bar = 2 μm.

(iii) by fluorescent staining, an acid pH within vacuoles enclosing rickettsiae which is identical to the pH in lysosomes of control uninfected cells. These findings conclusively show that fusion occurs between lysosomes and rickettsia-containing phagosomes in persistently infected J774 cells.

Despite P-L fusion, this organism survives and proliferates within the rather hostile environment of the phagolysosome. Other bacteria, including *S. typhimurium* and *M. lepraemurium*, have also been shown to proliferate within phagolysosomes of cultured macrophages (10, 18). It seemed logical to propose, as one of the reasons for rickettsial survival, an alteration in the hydrolytic functions of lysosomes in infected J774 cells. The results presented in this study demonstrate no significant differences in the activities of acid phosphatase and β-acetylglucosaminidase in infected and uninfected cells. β-Glucuronidase, β-galactosidase, and lysozyme activities, however, appeared to be slightly higher in infected cells.

Survival of *C. burnetii* in J774 cells may be due to the resistance of the organism (by an as yet unknown mechanism) to lysosomal hydrolases; this was also previously suggested by Burton et al. (7). Alternatively, *C. burnetii* may exhibit some susceptibility to the lysosomal hydrolases. Electron microscopic examinations in this study and previously (7, 8, 22) have revealed rickettsiae at what appear to be different stages of degradation in infected host cells.

Survival may be due to a proliferative poten-

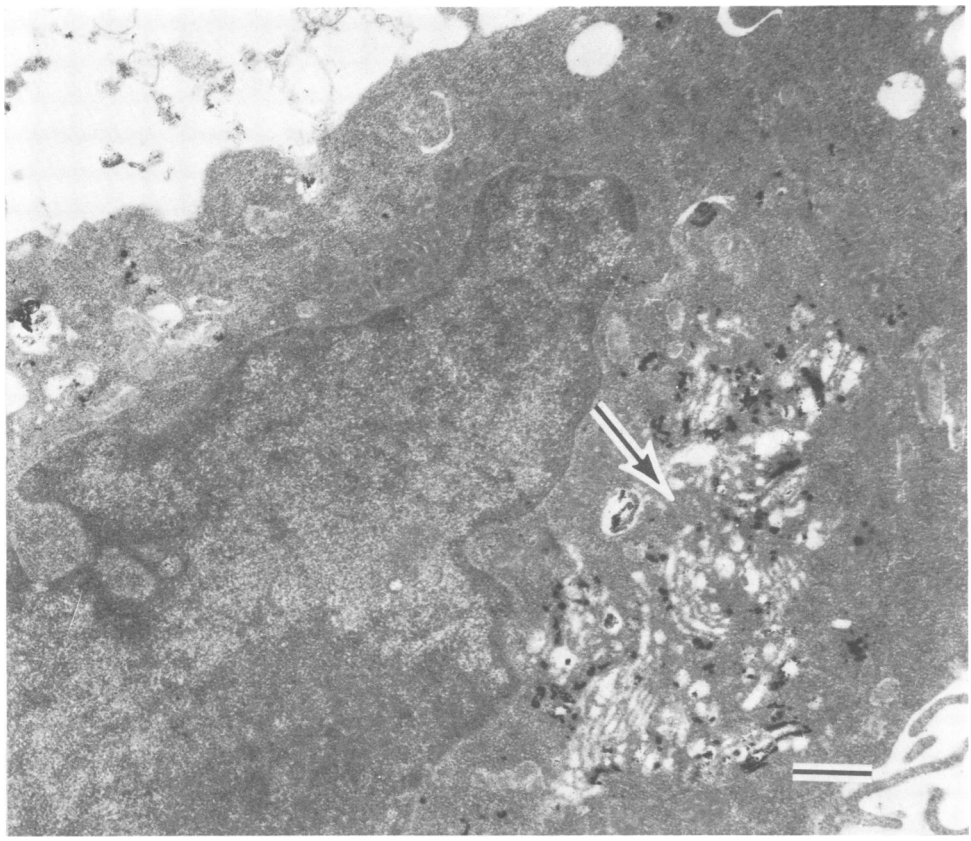

FIG. 4. Electron micrograph of a portion of the cytoplasm of an uninfected J774 cell cytochemically examined for acid phosphatase activity. The electron-dense lead phosphate reaction product is abundant in the region of the Golgi (arrow). Bar = 1.76 μm.

tial of *C. burnetii* that far exceeds the rate of destruction by lysosomal hydrolases (7). Further confounding the interpretation of the role of lysosomal enzymes in determining the fate of *C. burnetii* is the divergence in published reports (7, 8, 22) of the activities of lysosomal hydrolases after infection in different host cells. For example, Kishimoto et al. (22), in their in vitro study of the fate of normal and immune serum-treated *C. burnetii* in oil-stimulated guinea pig peritoneal macrophages, found that whereas ingested antibody-treated organisms were destroyed, normal serum-treated rickettsiae survived and proliferated within macrophage vacuoles. Analyses of the macrophage enzymes after 2 days in culture, however, revealed no significant alteration in the activities of β-galactosidase, β-acetylglucosaminidase, acid phosphatase, and lysozyme. Only β-glucuronidase activity was increased. Lysosomal hydrolases could not account for the differential fate of ingested rickettsiae. Burton et al. (7) reported an increase in acid phosphatase activity as determined by electron microscopic cytochemistry during short-term infections (1 to 2 h postinoculation) of L cells with *C. burnetii*; 5'-nucleotidase activity remained unchanged during this period. However, in persistently infected L cells maintained for 6 to 10 months in vitro, they reported a lack of detectable acid phosphatase activity in rickettsia-containing vacuoles (8). In contrast, acid phosphatase activity was elevated in persistently infected monkey kidney (Vero) cells (8).

More recently, Hackstadt and Williams (16) proposed a novel explanation to account for the establishment of *C. burnetii* in host cells. Survival and eventual proliferation were suggested to result from an acid pH activation of rickettsial transport and metabolism. By employing different lysosomotrophic agents (16) to alter the pH of *C. burnetii*-infected chicken embryo fibroblasts, it was suggested that an acid pH prevailed and was a requirement for rickettsial survival and replication within phagolysosomes. In this study the intraphagolysosomal pH of

TABLE 1. Lysosomal enzyme activity of uninfected and *C. burnetii*-infected J774 cells

Enzyme	Enzyme activity[a]	
	Uninfected	Infected[b]
Acid phosphatase	420.0 ± 80.0	460.0 ± 170.0
β-Acetylglucosaminidase	175.0 ± 19.0	187.0 ± 27.0
β-Galactosidase	46.0 ± 15.9	65.3 ± 22.9
β-Glucuronidase	606.7 ± 167.4[c]	773.3 ± 150.1
Lysozyme	0.73 ± 0.38[d]	1.04 ± 0.59

[a] Values are means of two determinations ± the standard error of the mean. Expressed as nanomoles of substrate hydrolyzed per 2.5×10^6 cells.

[b] Approximately 78 to 98% of the infected cell population contained at least 1 rickettsia per cell; 9 to 28% were heavily infected and contained ≥50 rickettsiae per cell.

[c] Value represents mean of three determinations ± the standard error of the mean and is expressed as nanomoles of substrate hydrolyzed per 5.0×10^5 cells.

[d] Micrograms of egg white lysozyme per 10^7 cells.

infected cells was actually measured. The phagolysosomal pH of *C. burnetii*-infected J774 cells determined from fluorescence measurements was ca. 5.2. This value is within the range reported for normal macrophage lysosomes (30).

Although the role of lysosomal hydrolases in determining the fate of intracellular parasites is still speculative, convincing evidence exists for the microbicidal role of reactive oxygen metabolites (23, 32). Recently, it was demonstrated that a J774 cell line variant (clone C3C) defective in oxygen metabolism (34) could kill or restrict the growth of normally replicating intracellular *T. cruzi* epimastigotes only after introducing an H_2O_2-generating system consisting of glucose oxidase-coated zymosan particles and glucose (34).

It appears that lysosomal hydrolases per se play a limited role (if any) in determining the fate of *C. burnetii* within the J774 cell line. The ultimate fate of an invading microorganism may depend primarily on the extent of the oxidative metabolic burst (i.e., generation of reactive oxygen metabolites) of the host cell during phagocytosis. Recently, Wilson et al. (35) demonstrated that human monocytes that generated reactive oxygen metabolites during phagocytosis of *T. gondii* were able to control the infectious agent. In contrast, human and mouse macrophages which produced little or no oxygen metabolites during phagocytosis of *T. gondii* were unable to kill or control its intracellular replication.

Studies are in progress to ascertain the status of oxygen metabolism in J774 cells upon exposure to and during prolonged infection with *C. burnetii*.

ACKNOWLEDGMENTS

This study was supported by grant PCM 8010633 from the National Science Foundation and Public Health Service grant RR-08139 from Research Resources, Minority Biomedical Support Program, National Institutes of Health.

LITERATURE CITED

1. **Armstrong, J. A., and P. D. Hart.** 1971. Response of cultured macrophages to *Mycobacterium tuberculosis*, with observations on fusion of lysosomes with phagosomes. J. Exp. Med. **134**:713–740.
2. **Armstrong, J. A., and P. D. Hart.** 1975. Phagosome-lysosome interactions in cultured macrophages infected with virulent tubercle bacilli. Reversal of the usual nonfusion pattern and observations on bacterial survival. J. Exp. Med. **142**:1–16.
3. **Axline, S. G., and Z. A. Cohn.** 1970. In vitro induction of lysosomal enzymes by phagocytosis. J. Exp. Med. **131**:1239–1260.
4. **Baca, O. G., E. T. Akporiaye, A. S. Aragon, I. L. Martinez, M. V. Robles, and N. L. Warner.** 1981. Fate of phase I and phase II *Coxiella burnetii* in several macrophage-like tumor cell lines. Infect. Immun. **33**:258–266.
5. **Beck, C., and A. L. Tappel.** 1968. Rat-liver lysosomal β-glucosidase: a membrane enzyme. Biochim. Biophys. Acta **151**:159–164.
6. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
7. **Burton, P. R., N. Kordova, and D. Paretsky.** 1971. Electron microscopic studies of the rickettsia *Coxiella burnetii*: entry, lysosomal response, and fate of rickettsial DNA in L-cells. Can. J. Microbiol. **17**:143–150.
8. **Burton, P. R., J. Stueckemann, R. M. Welsh, and D. Paretsky.** 1978. Some ultrastructural effects of persistent infections by the rickettsia *Coxiella burnetii* in mouse L cells and green monkey kidney (Vero) cells. Infect. Immun. **21**:556–566.
9. **Canonico, P. G., and J. W. C. Bird.** 1970. Lysosomes in skeletal muscle tissue: zonal centrifugation evidence for multiple cellular sources. J. Cell Biol. **45**:321–333.
10. **Carrol, M. E. W., P. S. Jackett, V. R. Aber, and D. B. Lowrie.** 1979. Phagolysosome formation, cyclic adenosine 3′:5′-monophosphate and the fate of *Salmonella typhimurium* within mouse peritoneal macrophages. J. Gen. Microbiol. **110**:421–429.
11. **Cummings, N. P., M. J. Pabst, and R. B. Johnston, Jr.** 1980. Activation of macrophages for enhanced release of superoxide anion and greater killing of *Candida albicans* by injection of muramyl dipeptide. J. Exp. Med. **152**:1659–1669.
12. **Densen, P., and G. L. Mandell.** 1980. Phagocyte strategy vs. microbial tactics. Rev. Infect. Dis. **2**:817–838.
13. **Fishman, W. H., B. Springer, and R. Burnetti.** 1948. Application of an improved glucuronidase assay method to the study of human blood β-glucuronidase. J. Biol. Chem. **173**:449–456.
14. **Gimenez, D. F.** 1964. Staining rickettsiae in yolk-sac cultures. Stain Technol. **39**:135–140.
15. **Gomori, G.** 1952. Microscopic histochemistry, principles and practice, p. 189–194. University of Chicago Press, Chicago, Ill.
16. **Hackstadt, T., and J. C. Williams.** 1981. Biochemical stratagem for obligate parasitism of eukaryotic cells by *Coxiella burnetii*. Proc. Natl. Acad. Sci. U.S.A. **78**:3240–3244.
17. **Hart, P. D.** 1981. Macrophage antimicrobial activity: evidence for participation by lysosomes in the killing of *Saccharomyces cerevisiae* by normal resident macrophages. Infect. Immun. **31**:828–830.

18. **Hart, P. D., J. A. Armstrong, C. A. Brown, and P. Draper.** 1972. Ultrastructural study of the behavior of macrophages toward parasitic mycobacteria. Infect. Immun. **5**:803–807.
19. **Jones, T. C., and J. G. Hirsch.** 1972. The interaction between *Toxoplasma gondii* and mammalian cells. II. The absence of lysosomal fusion with phagocytic vacuoles containing living parasites. J. Exp. Med. **136**:1173–1194.
20. **Karnovsky, M. L., and J. K. Lazdins.** 1978. Biochemical criteria for activated macrophages. J. Immunol. **121**:809–813.
21. **Kielian, M. C., and Z. A. Cohn.** 1980. Phagosome-lysosome fusion. Characterization of intracellular membrane fusion in mouse macrophages. J. Cell Biol. **85**:754–765.
22. **Kishimoto, R. A., B. J. Veltri, P. G. Canonico, F. G. Shirey, and J. S. Walker.** 1976. Electron microscopic study on the interaction between normal guinea pig peritoneal macrophages and *Coxiella burnetii*. Infect. Immun. **14**:1087–1096.
23. **Klebanoff, S. J.** 1975. Antimicrobial mechanisms in neutrophilic polymorphonuclear leukocytes. Semin. Hematol. **12**:117–142.
24. **Milder, R., and J. Kloetzel.** 1980. The development of *Trypanosoma cruzi* in macrophages in vitro. Interaction with lysosomes and host cell fate. Parasitology **80**:139–145.
25. **Nerurkar, L. S.** 1981. Lysozyme, p. 667–673. *In* D. O. Adams, P. J. Edelson, and H. Koren (ed.), Methods for studying mononuclear phagocytes. Academic Press, Inc., New York.
26. **Nogueira, N., and Z. Cohn.** 1976. *Trypanosoma cruzi*: mechanism of entry and intracellular fate in mammalian cells. J. Exp. Med. **143**:1402–1420.
27. **Ohkuma, S., and B. Poole.** 1978. Fluorescence probe measurement of the intralysosomal pH in living cells and the perturbation of pH by various agents. Proc. Natl. Acad. Sci. U.S.A. **75**:3327–3331.
28. **Ormsbee, R. A., and M. G. Peacock.** 1976. Rickettsial plaques assay and cloning procedures. Tissue Culture Assoc. Manual **2**:475–478.
29. **Phillips, H. J.** 1973. Dye exclusion tests for cell viability, p. 406–408. *In* P. F. Kruse, Jr., and M. K. Patterson, Jr. (ed.), Tissue culture methods and applications. Academic Press, Inc., New York.
30. **Reijngoud, D., and J. M. Tager.** 1977. The permeability properties of the lysosomal membrane. Biochim. Biophys. Acta. **472**:419–449.
31. **Rikihisa, Y., and S. Ito.** 1980. Localization of electron-dense tracers during entry of *Rickettsia tsutsugamushi* into polymorphonuclear leukocytes. Infect. Immun. **30**:231–243.
32. **Roos, D.** 1980. The metabolic response to phagocytosis, p. 337–385. *In* G. Weissman (ed.), The cell biology of inflammation. Elsevier/North-Holland Biomedical Press, New York.
33. **Silberman, R., and P. Fiset.** 1968. Method for counting rickettsiae and chlamydiae in purified suspensions. J. Bacteriol. **95**:259–261.
34. **Tanaka, Y., C. Kiyotaki, H. Tanowitz, and B. R. Bloom.** 1982. Reconstitution of a variant macrophage cell line defective in oxygen metabolism with a H_2O_2-generating system. Proc. Natl. Acad. Sci. U.S.A. **79**:2584–2588.
35. **Wilson, C. B., V. Tsai, and J. S. Remington.** 1980. Failure to trigger the oxidative metabolic burst by normal macrophages: possible mechanism for survival of intracellular pathogens. J. Exp. Med. **151**:328–346.